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Effect of obesity on thiol/disulfide balance in patients with polycystic ovary syndrome

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Abstract
Aim: In this study, we aimed to investigate the hypothesis that thiol/disulfide balance is similar in patients with PCOS with and without obesity.
Material and Methods: This was a prospective study. Seventy-eight patients with PCOS were included in the study. A diagnosis of PCOS was made according to the Rotterdam criteria. The patients were divided into two groups as obese [n = 41, body mass index (BMI) ≥30 kg/m^2] and non-obese (n = 37, BMI >18.5 and <30 kg/m^2).
Results: Native thiol and total thiol values were significantly lower in the oxidative stress test in the non-obese group than in the obese group (p = 0.021 and p = 0.019, respectively). There was no statistically significant difference in other thiol-disulfide parameters between the groups. Luteinizing hormone (r = -0.293, p = 0.09), total thiol (r = -0.321, p = 0.04), native thiol (r = -0.330, p = 0.03) and disulfide (r = -0.272, p = 0.16) rates were found to be statistically significantly negatively correlated with BMI.
Discussion: Obesity in PCOS affects thiol-disulfide hemostasis. There is a negative correlation between BMI and oxidative stress markers.

Keywords
Polycystic Ovary Syndrome, Obesity, Thiol, Oxidative Stress, Body Mass Index

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Introduction

Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders of the reproductive age, was first described by Stein and Leventhal in 1935 [1]. The syndrome causes many endocrine and metabolic disorders in the reproductive period and all periods of life. PCOS is a public health problem that also affects a person's quality of life. Obesity, which is a widespread problem today, often accompanies this syndrome. Different criteria are used to diagnose PCOS, and the prevalence of the disease varies according to the diagnostic criteria. According to Rotterdam criteria, the prevalence of PCOS in Turkey is 19.9% [2].

Oxidative stress increases in PCOS [3]. Redox sensitivity transcription factors regulate cell differentiation and apoptosis. In PCOS, due to the mildly increased inflammatory response, adequate antioxidation cannot be provided, and cellular necrosis develops. In PCOS, which is insulin resistant, insulin receptor substrate decreases and glucose uptake in muscle and adipose tissue is impaired. In cases of increased oxidative stress, intracellular calcium balance is also disturbed, mitochondria failure develops, ATP synthesis is distressed, resulting in follicular collapse. Oxidized proteins lose their function as proteins and can act as proinflammatory mediators. In addition, fatty acids in plasma and organelle membranes are transformed at a higher rate in PCOS. DNA oxidation markers are shown to be high in PCOS.

When DNA oxidation increases and the anti-oxidation mechanism cannot function adequately, transformation into cancer also increases [4].

Oxidative stress is high in obesity. Cytokines that accumulate in adipose tissue cause a proinflammatory reaction, which increases oxidative stress [5].

Thiol-disulfide homeostasis is one of the indicators of oxidative stress.

Native thiol binds with sulfide, oxidizes, and forms disulfide. In response to this, with the work of the antioxidant mechanism, sulfide bonds are separated and turn into native thiol again. The total thiol level is equal to the sum of native thiol and disulfide [6]. In thiol-disulfide tests, native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios are evaluated. Thus, oxidative stress increases with increasing disulfide concentration. Many studies are examining the oxidative stress balance in the current literature. However, a gold standard test has yet to be demonstrated.

In the last few decades, there has been an increase in studies examining oxidative stress levels with thiol-disulfide hemostasis. Erel et al. developed an inexpensive and easy calorimetric method that examined thiol-disulfide hemostasis. This method is frequently used in modern research. In the current literature, studies are examining oxidative stress levels in PCOS. However, the studies examining the effect of obesity on the oxidative stress level in PCOS are not sufficient. We planned this prospective, controlled study to support the literature on this topic.

In our study, we aimed to investigate the hypothesis that thiol/disulfide balance is similar in patients with PCOS with and without obesity.

Material and Methods

Study design

The study population was formed from patients who presented to Kırşehir Training and Research Hospital Gynecology and Obstetrics Outpatient Clinic between March 2020 and September 2020. Seventy-eight patients who were diagnosed with PCOS were included in the study.

Inclusion criteria

The diagnosis of PCOS was made in the presence of at least two of the Rotterdam criteria [7]. Rotterdam criteria: (1) Oligo- or anovulation, (2) Clinically and/or biochemically diagnosis of hyperandrogenism, (3) Polycystic-looking ovaries.

Exclusion criteria

(1) Congenital adrenal hyperplasia, (2) androgen-secreting tumor, (3) Cushing syndrome, (4) prolactinoma, (5) thyroid disorders, (6) known infectious diseases, (7) known chronic inflammatory diseases, (8) pregnancy, (9) chronic systemic disease (such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease, liver or kidney dysfunction), and (10) body mass index (BMI) <18.5 kg/m2.

Sample collection

Gynecologic examinations and height-weight measurements of the included patients were made, BMI calculation weight (kg) / (height) 2 (m2). The patients were divided into two groups as obes (n = 41, BMI ≥30 kg/m^2) and non-obese (n = 37, BMI >18.5 and <30 kg/m^2) (Organization WHO. Guide for the formulation of a WHO Country Cooperation Strategy. 2014).

Sample preparation and laboratory analysis

Venous blood sampling of the patients was performed on days 3-5 of their spontaneous or induced menstrual cycles.

Hormone analysis

Levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, and dehydroepiandrosterone sulfate (DHEA-s) were examined in serum using a Cobas e801 analyzer (Roche Diagnostics, Germany). C-reactive protein (CRP) was analyzed using a Cobas c702 (Roche Diagnostics) autoanalyzer. Homeostatic model assessment-insulin resistance (HOMO-IR) was calculated using the following formula:

Insulin × glucose / 405 mg/dL.

Thiol- disulfide analysis

Venous blood samples were also centrifuged at 3000 rpm and stored at -80°C until the analysis date. Thiol/disulfide homeostasis was evaluated using the spectrophotometric method developed by Erel et al. [6].

Ethical considerations

This prospective study was planned according to the current Helsinki criteria. Ethical approval for the study was obtained from Kırşehir Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee (Decision number 2020-19/141).

Statistical analysis

Statistical analyses were performed using the SPSS version 25 software package (SPSS In., Chicago, IL, USA). Whether continuous variables had normal distribution was analyzed using the Kolmogorov-Smirnov test, and the homogeneity of variables was analyzed using the Levene test. Student's t-test was used for the comparison of the groups. Correlations between variables were evaluated using Pearson's correlation

coefficient. P values less than 0.05 were considered statistically significant. Data are given as mean ± standard deviation (SD).

Results

Thirty-seven obese and 41 non-obese patients with PCOS were included in the study. The mean age of the two groups was 23.86 ± 5.00 years for the obese group and 22.00 ± 3.85 years for the non-obes group. The mean BMI was 36.21 ± 5.47 kg/m² in the obese group and 23.85 ± 3.27 kg/m² in the non-obese group.

Table 1. Comparison of blood values between the two groups

	Obese (n=37)	Nonobese (n=41)	p
LH mIU/mL	10.64±6.31	17.48±11.84	0.002
FSH mIU/mL	5.54±2.40	5.59±1.85	0.908
HOMA-IR mg/dL	4.53±3.10	3.03±2.02	0.012
Testesteron ng/dL	0.43±0.20	0.58±0.52	0.111
DHEA-s mmol/L	274.89±120. 97	295.04±155. 57	0.528
CRP mg/L	0.78±0.69	0.30±0.32	0.000

Data presented as Mean ± SD. Student's t-test was used for the comparison of the groups. LH: Luteinizing hormone. FSH: Follicle stimulating hormone. HOMA-IR: Homeostatic model assessment-insulin resistance. DHEA-s: Dehydroepiandrosterone sulfate. CRP: C -reactive protein.

Table 2. Thiol/disulfide balance in obese and non-obese PCOS patients

	Obese (n= 37)	Nonobese (n=41)	p
Native thiol (µmol/L)	279.72±24.87	292.51±23.32	0.021
Total thiol (µmol/L)	330.40±31.15	346.65±28.61	0.019
Disulfide (µmol/L)	25.32±4.22	27.09±3.60	0.059
Disulfide/native thiol (%)	8.97±1.21	9.29±1.05	0.217
Disulfide/total thiol (%)	7.62±0.98	7.80±0.81	0.370
Native thiol/total thiol (%)	84.68±1.68	84.49±1.59	0.615

Data presented as Mean ± SD. Student's t-test was used for the comparison of the groups.

Comparison of blood values between the two groups is given in Table 1. LH was significantly higher in the non-obese group (p=0.002). HOMA-IR, there was a statistically significant difference between the two groups (p = 0.012). There was no significant difference between FSH, total testosterone, and DHEA-s (p = 0.908, p = 0.105, p = 0.604).

Table 2 shows the thiol-disulfide results of the groups. Native thiol and total thiol values were significantly lower in the oxidative stress test group in the non-obese group than in the obese group (p = 0.021 and p = 0.019, respectively). There was no statistically significant difference in other thiol-disulfide parameters between the groups.

Table 3 shows the results of the correlation analysis of thiol/disulfide values with BMI and hormone parameters. LH (r = -0.293, p = 0.09), total thiol (r = -0.321, p = 0.04), native thiol (r = -0.330, p = 0.03) and disulfide (r = -0.272, p = 0.16) rates were found to be statistically significantly negatively correlated with BMI. There was a positive correlation between HOMA-IR and CRP (r = 0.393, P < 0.001) and BMI (r = 0.347, p = 0.002). A negative correlation was found between CRP and total testosterone (r = -0.260, p = 0.22), DHEA-s (r = -0.286, p = 0.012), BMI (r = -0.229, p = 0.045), native thiol (r = -0.229, p = 0.045), disulfide/native thiol (r = -0.260, p = 0.22), disulfide/total thiol (r = -0.286, p = 0.12), native thiol/total thiol (r = -0.237, p = 038).

Discussion

This study compared thiol-disulfide hemostasis and we found that native and total thiol among oxidative stress markers in obese patients with PCOS were lower than in non-obese patients. There was a negative correlation between native thiol, total thiol, and disulfide ratios and BMI.

Özler et al. compared thiol-disulfide homeostasis between two

Table 3. Correlation analysis of thiol/disulfide values with BMI and hormone parameters

		LH	FSH	T	DHEA-s	BMI	HOMA-IR	CRP
Native thiol	R	.042	.074	.010	-.036	-.330**	.042	-.229*
	P	.715	.520	.932	.757	.003	.715	.045
Total thiol	R	.053	.066	-.010	-.034	-.321**	.053	-.187
	P	.646	.567	.933	.769	.004	.646	.103
Disulfide	R	-.002	.081	.064	.044	-.272*	-.002	.218
	P	.985	.479	.578	.703	.016	.985	.057
Disulfide/native thiol	R	.032	-.102	-.112	.086	.091	.032	-.260*
	P	.780	.373	.331	.454	.016	.780	.022
Disulfide/total thiol	R	-.049	.022	.081	.015	-.162	-.049	-.286*
	P	.671	.848	.479	.896	.155	.671	.012
Native thiol/total thiol	R	-.034	.019	.144	.008	-.183	-.034	-.237*
	P	.764	.870	.209	.948	.109	.764	.038
BMI	R	-.293**	-.054	.191	-.086	1	.347**	.393**
	P	.009	.636	.094	.455		.002	.000
HOMA-IR	R	-.158	-.115	-.158	-.032	.347**	1	.261*
	P	.167	.315	.167	.783	.002		.022
CRP	R	-.187	.218	-.260*	-.286*	-.229*	.393**	1
	P	.103	.057	.022	.012	.045	.000	

**Correlation is significant at the 0,01 level. *Correlation is significant at the 0,05 level. Correlations between variables were evaluated using Pearson's correlation coefficient.
BMI: Body Mass Index. CRP: CRP: C-reactive protein.LH: Luteinizing hormone. FSH: Follicle stimulating hormone. T: Testosterone HOMA-IR: Homeostatic model assessment-insulin resistance. DHEA-s: Dehydroepiandrosterone sulfate.

groups with and without obesity in adolescents with PCOS. In this study, native and total thiol levels were significantly lower in patients with obesity, similar to our findings [8]. In addition, in our study in the adult group, disulfide levels were negatively correlated with BMI. In another study, the level of glutathione S-transferases (GSTs) was examined as an oxidative stress test. According to this study, the antioxidant mechanism in adolescents with PCOS is independent of obesity [9]. In our study, there was a significant negative correlation between oxidative stress factors and BMI. Yıldırım et al. compared patients with PCOS and a normal healthy group and concluded that antioxidant markers were higher in the non-obese group [10]. Our study compared obese and non-obese groups with PCOS and we found significant differences only in native and total thiol levels.

PCOS and oxidative stress levels have been evaluated with different tests and different results have been determined. Tola et al. evaluated neopterin (NEO) levels as a stress index in PCOS, and a significant elevation was found [11]. However, Dereli et al. examined serum malondialdehyde levels in patients with PCOS and found no significant difference between the normal population and those with PCOS [12]. Our study found that some antioxidative parameters were low in the obese PCOS group.

CRP increases due to inflammation in PCOS and obesity [13]. Elmas et al. found that CRP was higher in the obese group than in the non-obese group in children [14]. There was a negative correlation between BMI and antioxidant parameters [15]. In our study, we found a similar result in adults with PCOS.

We found that there was a negative correlation between LH and BMI. Pergola et al. stated that the increase in BMI suppressed gonadotropin secretion and had a negative correlation with LH and BMI [16]. Inflammatory factors are activated in PCOS and obesity. Insulin resistance also runs parallel with increasing BMI. Our study showed a positive correlation between HOMA-IR and CRP and BMI, which was consistent with the literature [17, 18]. A standard treatment option for PCOS cannot be offered because its etiopathogenesis has not yet been elucidated. Enlightening this mechanism will increase our treatment success. Oxidative stress may be the underlying disorder of PCOS or the result of the disease. Until the relationship between obesity and PCOS is clarified, larger case series and studies comparing different methods are required.

Limitations

Normal threshold values for thiol-disulfide parameters have not been established yet, and large series studies are needed for this.

Conclusion

Obesity in PCOS affects thiol-disulfide hemostasis. There is a negative correlation between BMI and oxidative stress markers.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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